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Personal Report

Howard Holtzer – Developmental and cell biologist 1922–2014

Few developmental biologists have had as great an impact on the conceptual thinking of those in their field as Dr. Howard Holtzer. Howard's three greatest gifts were his ability to make penetrating microscopic observations, his conceptualization of biological processes, and his ability to identify and train those who became prominent scientists. While frequently personally controversial, and often outspoken, his observations and concepts influenced his detractors as well as his admirers. Over 200 publications convey his evolving ideas of cell differentiation and development, and demonstrated the remarkable breadth of his contributions as well as the intellectual excitement he provoked. Noteworthy are his contributions to the concept of cell lineage and the importance of cell division in the delineation of cell diversity; his insights into induction; and his discovery and perception of the importance of intermediate filaments and cytoskeletal elements in differentiation and assembly. Howard worked in many developmental systems including the nervous system, cardiac and skeletal muscle, cartilage, pigment, and hematopoietic tissues, discerning the common elements that unified differentiation and embryonic development. His contributions and the influence he had are reflected in the large number of productive scientists that he trained.

Howard was a Professor of Cell and Developmental Biology, Emeritus, at the University of Pennsylvania when he died at his home in Philadelphia on November 5, 2014. He was 92 years old. He was born in Brooklyn and served in the Army in the Pacific Theater during World War II. He received his B.S. in 1947 and Ph.D. in 1952 at the University of Chicago in the laboratory of Paul Weiss. Following a postdoctoral fellowship at the College of Physicians and Surgeons, Columbia University, with Samuel Detwiler, who had received his Ph.D. with Ross Harrison at Yale, Howard joined the faculty at the University of Pennsylvania in 1953. Howard and his mentors followed in the footprints of the most prominent thinkers in Embryology in the first half of the 20th century.

Howard was as much a theoretical as he was an experimental biologist. In his early work he investigated the classic embryological problem of cell differentiation. This led him to postulate a gradual revelation of the differentiated state within embryonic cells separated by a series of cell replication steps that he viewed critical as development proceeded. He was among those early on who stressed the concept of lineage in developing organism where cells became entrained in a pathway by events that were associated with DNA synthesis. In mid-career he turned to analysis and description of the importance of intracellular filaments where he and his colleagues made one of his most important discoveries, intermediate filaments.

Howard's initial work was published in an era of developmental and cell biology that was rapidly transitioning from the descriptive to the mechanistic because of a flurry of new technology. His published dissertation with Paul Weiss in 1951 was on regeneration of the spinal cord and formation of the vertebral column in the salamander. This dissertation and then work as a post doc with Detwiler, established his enduring interest in chondrogenesis and myogenesis. Using extirpation and transplantation techniques in vivo in the early 1950s, he established that the spinal cord and the notochord were required for the initiation of vertebral cartilage formation and axial muscle formation by induction through tissue–tissue interactions (Holtzer and Detwiler, 1953). This work was quickly extended from in vivo assessment, to organ culture, and then cell culture with his first faculty appointment at the University of Pennsylvania in 1953.

Howard was among a burgeoning group of young embryologists (soon to be called developmental biologists) and cell biologists exploiting new technologies emerging in the late 1950s and early 1960s. This was the hay day of descriptive embryology just beginning to make the transition to the use of biochemistry and the emerging tools of molecular biology and genetic models in the exploration of developmental mechanisms. The Journal of Developmental Biology had just been founded in 1959; Grobstein had just demonstrated inductive interactions between epithelium and mesenchyme in transfilter organ culture; this was followed by collaborations with Rutter using biochemistry to identify minute amounts of differentiated cell product; in a brief paper Moscona had described tissue dissociation using enzymatic digestion that

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accelerated cell culture; Konigsberg had begun to culture monolayers of muscle cells and cell cloning; and Hayflick was exploring the role of cell proliferation in maintenance of the diploid state. These approaches were central to all of Howard's work including his first major contribution. Using organ culture techniques, his technician, Joan Abbott, his wife and collaborator, Sybil and his postdoctoral fellow, Jay Lash, extended Howard's graduate work to demonstrate in vitro the induction of chondrogenesis by the spinal cord. This work established a diffusible factor(s) was involved in chondrogenesis of the ventral column. This was the first time that biochemical techniques were applied to understand induction of chondrogenesis. This work lay the grounding for our current understanding of the inductive roles of signaling molecules disseminated by the ventral neural tube to initiate formation of the vertebral column.

Myogenesis was the foundation of much of Howard's important experimental and theoretical contributions. Working at the Carlsberg Laboratories in Denmark, Howard, working with John Marshall and Henry Finck, demonstrated the power of using fluorescein labeled polyclonal antibodies to investigate early events in cell differentiation, specifically the assembly of nascent sarcomeric myosin within embryonic muscle fibers (Holtzer et al., 1957). The work with Marshall lead to one of the first quantitative molecular descriptions of early differentiated cell function. Immunohistochemistry has become a mainstay in biological studies of cell differentiation and in many fields of biology and medicine.

The mechanism for the formation of large multinucleated muscle cells during embryonic development and muscle regeneration had been a debated subject for nearly two centuries. The advent of cell culture techniques permitted the Holtzer laboratory to pursue the biology of myogenesis. With Jay Lash and Hewson Swift, he demonstrated that every nucleus in a fiber had a diploid amount of DNA (Lash et al., 1957); leading to the conclusion that replication of nuclei within muscle fibers is not the process leading to multinucleation. This work was then extended to cell culture by his students demonstrating that nuclei of developing muscle fibers never synthesized DNA in the transition to multinucleation, leading to the conclusion that myogenic cells fuse to form multinucleated fibers, indicating that DNA synthesis and differentiation were mutually exclusive processes. These observations spawned Howard's theories on cell differentiation that he pursued over the rest of his career. His most well-known idea was “quantal” versus proliferative mitoses (Holtzer et al., 1972). In more general form, Howard thought the central problems of cell differentiation related to mechanisms where by cells acquire from their immediate progenitor cells the machinery to respond to a variety of unspecific inducers so as to produce specific molecules characteristic of the differentiated cell. This central problem relates to those endogenous mechanisms that make available in daughter cells genetic information that was not readily available in the mother cell. Therefore he thought differentiation is best thought of as the process by which daughter cells emerge those synthetize molecules their mother cells did not. When cell divisions yield daughter cells that duplicate the mother cell's phenotype, he designated these as proliferative, and those that have new pathways, as quantal cell cycles (Holtzer et al., 1972).

The theoretical consideration of the role of cell lineage in development was a forerunner of the importance of stem cells in developmental, regenerative, and maintenance functions of organisms especially in skeletal myogenesis. While the satellite cell on muscle fibers was first described in 1961 by Mauro, the ideas and observations of Howard and others advanced Mauro's suggestion that satellite cells must be stem cells responsible for myogenesis and regeneration. Howard's theories on cell lineage were incorporated into the work of Groudine and Weintraub, then graduate students in Howard's laboratory, in the analysis of hematopoiesis.

The concept of the “quantal mitosis” captured the imagination of all those interested in myogenesis from the mid-1970s to the mid-1980s. He postulated an obligatory role of DNA synthesis in reprograming precursor myogenic cells to produce terminally differentiated cells in the myogenic lineage. This theory led to many publications throughout the world attempting to validate or discredit the idea. His concept of the quantal mitosis evolved over time, but always embodied the idea of a cell lineage, where progressive cell cycles made available regions of the genome that were not available for transcription in the mother cell such that the daughter cell at a particular cell cycle manifest the differentiated state (Holtzer et al., 1972). While fruitful both in Howard's laboratory and in the subsequent seminal work of Weintraub in his identification of MyoD as a master regulator of myogenesis, the idea of quantal mitosis began to fade in the late 1980s as work on myogenic regulators showed that many cell types could be converted to myogenic cells by transfection of a single gene, work that anticipated the recent discovery of reprogramming genes.

Howard's interest in myogenesis led to his discovery of intermediate filaments. This discovery was rooted in observations made with Marshall and Finck using immunohistological assays of assembly into myofibrils in embryonic muscles (Holtzer et al., 1957). They observed that the first myofibrils formed beneath the cell membrane, or sarcolemma. Howard proposed that the first myofibrils serve as templates for the formation of additional myofibrils and that stress-fiber-like structures might serve as a template for the formation of the first myofibrils (Dlugosz et al., 1984). In their seminal study in 1968, Ishikawa, Bischoff and Holtzer measured the diameters of the filaments in the developing muscle cells in culture (Ishikawa et al., 1968) and found the presence of three types of filaments: actin filaments, myosin filaments and a group of filaments with a diameter of 100 Å, intermediate between those of the 60 Å thin actin filaments and the 150 Å thick myosin filaments. These “intermediate filaments” appeared first in bundles running along the long axis of the early myotube. Other cell types, such as fibroblasts, epithelial cells, and chondrocytes also had these filaments. This classic 1968 paper was selected as a Landmark Review by the journal of NIH Research in 1996 and is the most cited paper of the approximately 200 publications of Howard Holtzer and his collaborators. Their subsequent work identifying polarity of actin filaments in muscle and onnon-muscle cells was foundational in understanding mechanisms of cell motility (Ishikawa et al., 1969).

Howard was intensely passionate about science and he held strong views that impacted his teaching, his personal relationships, his involvement in social issues, and his scientific presentations. Some found his presentations abrasive, which diminished the recognition he deserved. But his approach produced work that was frequently published in the leading journals and influenced a generation of scientists. He was a committed and effective mentor who stimulated his trainees to question experimental findings, to generate alternative hypothesis to explain their findings and those of others, and to challenge widely held views in developmental and cell biology. His approach was successful, as evidenced by the numbers of medical students who joined the ranks of academic medicine to pursue basic science rather than clinical investigation, and the numbers of graduate students and post docs who pursued successful careers. Howard was especially proud of the legacy of students he trained and their successes. In his later years, Howard continued to be energized by his interactions with students and fledgling faculty, with whom he regularly engaged in passionate discussions on current scientific ideas and emerging data. He and Sybil also fostered social causes, particularly in their early years in Philadelphia, and they spoke out about injustices done to fellow scientists. And he was a frequent international guest investigator,

particularly in Japan where for many years he carried on fruitful collaborations.

Howard Holtzer remained scientifically active and engaged to the end of his life, having a scientific career of 64 years, publishing his last paper at the age of 87. He was predeceased by his daughter, Celia, and is survived by his wife and life-long scientific collaborator, Sybil, who was his companion in all things.

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